Chemistry and Materials Research
ISSN 2224- 3224 (Print) ISSN 2225- 0956 (Online)
Vol.9 No.1, 2017

www.iiste.org

Investigation into Trihalomethanes in Lager Beers Brewed in Ghana

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Abstract
Water is a vital ingredient in beer constituting 91-98% of the content. In Ghana, the main source of water for the two major breweries in the city of Accra is from the Ghana Water Company which takes its source from the Weija Dam and the quality of the water, which may be changed without warning. Moreover, chlorination is the most widely used water disinfection process. Trihalomethanes are formed when water containing natural organic matter reacts with chlorine used in disinfection and other halogens. Trihalomethanes are potential carcinogens and it has been reported to cause a higher incidence of bladder and colon cancer in animals. Therefore, this research was carried out to determine the levels of trihalomethanes in six selected brands commercially brewed lager beers by the two major breweries in Ghana. A gas chromatograph equipped with an electron capture detector coupled with a headspace auto-sampler fitted with the column HP-5, 30m × 0.32mm × 0.25µm was used in analyzing fourty-eight bottles of lager beers comprising of 8 different batches from each of the six brands. The highest trihalomethane detected in the lager beers was chloroform with a concentration 20.19 ± 2.73µg/l and the least detected was dibromochloromethane of concentration 0.282 ± 0.06µg/l. The levels detected were below the World Health Organization's total daily intake guideline values of 300µg/l, 60µg/l, 100µg/l and 100µg/l for chloroform, bromodichloromethane, dibromochloromethane and bromoform respectively in drinking water. However, regular intake of beer with higher concentrations of trihalomethanes as it was observed in some of the samples can increase the risk of one being diagnosed of cancer related ailments in the longer future. It is therefore recommended that the regulatory bodies such as the Food and Drugs Authority (FDA) and the Ghana Standards Authority (GSA) must begin to monitor the levels of trihalomethanes in beers and other beverages that are produced in the country to help safeguard the health of the citizenry.
Keywords: Trihalomethanes , carcinogens, chlorination, gas chromatograph, lager beers

Introduction

Beer happens to be one of the beverages of choice for the Ghanaian during social gatherings and occasions such as marriage ceremonies, naming ceremonies, funerals, Christmas festivities, Easter celebrations, festivals and so on and so forth.

Beer demand is rising rapidly – at a rate of 5-10% annually by volume. The current Ghana market size is estimated at 1.65 million hectolitres, the equivalent of almost 7 litres per head of population according to drinksinfo.wordpress.com (2013). This indicates that beer consumption among the citizenry is on the increase.

Water, in terms of quantity, is the most important raw material of beer (Krottenthaler & Glas, 2009). The chemical and biological composition of water therefore has a significant relevance in beer production, and there is no step in the brewing process that is not influenced by the constituents of water (Krottenthaler & Glas, 2009).

According to Briggs et al (2004) beers usually have water contents of 91%-98% and breweries may receive water from different sources, which may be changed without warning.

In Ghana, the main source of water for the two major breweries in the city of Accra is from the Ghana Water Company which takes its source from the Weija Dam; surface water situated on the Accra-Kasoa highway and for that matter the Weija treatment plant.

Trihalomethanes are small organic compounds similar in structure to methane, but they have three hydrogen atoms substituted with chlorine or bromine (Carpi & Zufall, 2003). Chloroform is the most commonly occurring trihalomethane (THM). They are formed in water when disinfectants such as the chlorine used in water-treatment plants react with organic matter; for example, humic acids, which are found in the source water, especially in case of surface waters (Carpi & Zufall, 2003). Humic acids are the organic portion of soil formed by the decay of leaves, wood, and other plant materials (Carpi & Zufall, 2003). Disinfectants reduce the levels of microbes in the water supply; however, as the use of disinfectants in water increases, the risk of formation of trihalomethanes increases. According to Briggs et al (2004) organic materials are particularly likely to be present in surface waters and may be dissolved or present as colloidal or suspended materials. Humic and fulvic acids are crude mixtures of organic materials with molecular weight ranges of 500-2,000,000 and 200-1,000 respectively. These are particularly likely to give rise to THMs during chlorination. The bromine substituents can be added when the chlorinated water contains bromide ions. The composition of the THM group varies. It includes chloroform, bromomethane, carbon tetrachloride, 1,1-dichloroethane, 1,1,2-trichloroethane and tetrachloroethane together with a range of other substances. Thus, trihalomethanes can be found in most disinfected drinking water supplies. The most important trihalomethanes in disinfected water are chloroform, dichlorobromomethane, chlorodibromomethane, and bromoform (Carpi & Zufall, 2003).

Studies of human populations have indicated a slightly higher incidence of bladder and colon cancers in areas where the drinking water has been chlorinated (Carpi & Zufall, 2003). Other studies show the association of waterborne chloroform in drinking water with low birth weight, prematurity and intrauterine growth retardation (Carpi & Zufall, 2003). Animal studies have found possible relationships between oral exposure to various trihalomethanes and fetotoxicity and sperm abnormalities (Carpi & Zufall, 2003).

Again liquor used in the brew house loses most of these undesirable organic constituents during the boiling stage. However, the blending liquor (deaerated water), used to dilute beers brewed at a high gravity down to sales strength or bottle specification, is not heated, and therefore requires more careful checking and treatment according to Wu et al (2006).

There is no specific legal limit for TTHMs in food and drink. The World Health Organization (WHO) stipulates that the sum of the ratio of the concentration of each trihalomethane to its respective guideline value should not exceed 1. The WHO guideline values for Chloroform, bromoform, bromodichloromethane and dibromochloromethane are 300ug/L, 100ug/L, 60ug/L and 100ug/L respectively in drinking water (WHO, 2008).

The U.S. Environmental Protection Agency (EPA) has published the Stage 1 Disinfectants and Disinfection By-products Rule to regulate total trihalomethanes at a maximum allowable annual average level of 80 ppb. In December 2001, this standard replaced the 100-ppb maximum allowable annual average level for large surface public water systems.

Trihalomethanes, THMs are potential carcinogens and it has been reported to cause a higher incidence of bladder and colon cancer in animals (Carpi & Zufall, 2003). Moreover it leads to premature births, low birth weight, intrauterine growth retardation as well as fetotoxicity and sperm abnormalities (Carpi & Zufall, 2003).

The various water treatment processes in breweries such as gas stripping, activated carbon filtration and reverse osmosis can eliminate the THMs in the brewing liquor. Also, brewing processes such as mashing and wort boiling eliminates THMs since they are volatile. However, the activated carbon filters when used may become inefficient in removing the THMs when it takes a longer time for back washing and regeneration leading to some THMs finding their way into the brewing liquor and the final beer.

However, the frequency of backwashing and regeneration of the activated carbon filters can make them inefficient in eliminating THMs in situations where those processes are delayed.

Again, the dilution liquor which is used in bringing high gravity brews into bottle specifications is not heated and as a result of this should the water contain THMs, they are potentially going to find their way into the final product (Wu et al, 2006).

In Ghana however, there is little record of determination or measurement of THMs in the municipal water used by the major breweries in brewing their various brands of beer. It is also not clear whether or not in the breweries; the municipal water is further treated as is done elsewhere to remove any potential THMs.

Lastly, if the breweries do treat the water further to remove potential THMs, how effective is the process in removing the THMs from the water before it is used in the brewing process?

Therefore, the question arises whether there are THMs present in the brewing liquor and lager beers produced in Ghana and if they are present are the levels within the WHO guideline values?

The aim of this research was to determine whether THMs were present in lager beers produced in Ghana and also whether the levels present were within the WHO guideline values.

Methodology

CHEMICALS AND STANDARDS

Trihalomethane calibration mix was supplied by Sigma-Aldrich (Supelco, Bellefonte, PA, USA) as ampoules of 1ml of concentration 100µg/ml containing $CHCl_3$, $CHCl_2Br$, $CHClBr_2$ and $CHBr_3$ respectively in methanol. Analytical grade sodium hydroxide (NaOH), Sodium chloride (NaCl), sodium thiosulphate ($Na_2S_2O_3$) and Nitric acid were supplied by Merck (Darmstadt, Germany).

Stock trihalomethane calibration mix standard was prepared by diluting 1 ampoule to 100ml using deionized water prepared from a Millipore deionizer, Milli-Q$^{TM.}$ Working solutions of 1ppb, 20ppb, 50ppb, 70ppb and 100ppb were then prepared by diluting various volumes of the stock solution. The working solutions were used for the calibration of the gas chromatograph. The stock solution and the working solutions were stored in a refrigerator at a temperature of 4^oC. 6M NaOH solution was prepared by dissolving 60g of NaOH in deionized water and diluting it up to 250ml mark in a volumetric flask. This solution was used in neutralizing the carbonic acid (pKa 6.1) in the beer samples due to the carbonation. 10% $Na_2S_2O_3$ solution was prepared by diluting 10g of the chemical in a 100ml volumetric flask. This solution was added to water samples to prevent any oxidative effect of chlorine on organic matter during storage.

A 10% nitric acid solution was also prepared by diluting 57.14ml of 70% nitric acid to 400ml using deionized water. This solution was used in cleaning all glass wares prior to their use in the analyses.

BEER SAMPLES

Six different lager beers produced by the two major breweries in the country were sampled one(1) per week over a period of 8 weeks. This was done to ensure that different batch numbers and for that matter samples produced on different days were sampled. Thus 48 samples altogether were purchased from pubs and retail shops across the city of Accra within the 8weeks period and stored at room temperature before being analysed.

SAMPLE PREPARATION

The brewing liquor and dilution liquor samples were prepared by pipetting 10ml sample into a 20ml GC grade vials containing 4g of NaCl and immediately covering them with the crimp caps. The vials were then vortex at a speed of 500rpm for 1minute to dissolve the salt prior to analysis. This was done to increase the ionic strength of the sample, leading to a change in the vapor pressure, viscosity, solubilities of solutes, density, and surface tension, and resulting in altered liquid/vapor equilibria of the analytes in the system.

The beer samples were prepared by pipetting 10ml into a 20ml GC grade vial containing 300µl of 6M NaOH (to neutralize the carbonic acid present) and 4g of NaCl (to increase the ionic strength) after which the crimp caps were fixed in place. The vials were then vortex at a speed of 500rpm for 1minute to dissolve the NaCl prior to the GC analysis. The vials were then placed in the auto-sampler carousel and analyzed based on the operating conditions stated under the instrumentation section.

INSTRUMENTATION AND CALIBRATION

All chromatographic analyses were done using a gas chromatograph 7890B model (Agilent Technologies, Palo Alto, CA, USA) equipped with an electron capture detector (ECD) and fitted with a headspace auto-sampler 7697A model (Agilent Technologies, Palo Alto, CA, USA). The column for chromatographic separation was HP-5, 30 m × 0.32 mm × 0.25 µm. The headspace auto-sampler operating conditions were as follows: vial equilibration time, 30 min; oven temperature, 60°C; vial pressurization time, 8s; loop fill time, 9s; valve/loop temperature, 70°C. Ultra-pure nitrogen (99.999% purity supplied by Air Liquide, Tema, Ghana) was used both to pressurize vials and to drive the headspace formed to the injection port of the chromatograph via a transfer line at a temperature of 80°C. Injection was done in the split mode (split ratio 1:1) with an inlet temperature of 90°C; flow rate of carrier gas (N_2) was fixed at 8mL/min. The gas chromatographic separation was achieved on an HP-5MS UI fused silica capillary column, (30 m × 0.32 mm × 0.25 µm) coated with a stationary phase consisting of 5% phenyl, 95% methylpolysiloxane supplied by Agilent. Oven temperature was programmed as follows: Initial temperature 70^{o}C for 1 min and ramped at 20°C/min to 130°C, and finally held for 2.2 min. The detector temperature was set at 150^{o}C and that of the injector was 90^{o}C.

A multilevel calibration was used with 5 levels and 4 compounds. Five different working solutions (approximately 1µg/l, 20µg/l, 50µg/l, 70µg/l and100µg/l) were prepared from the trihalomethanes calibration mix. Three (3) vials of calibration standard from each of the working solutions were prepared by pipetting 10ml of the standard into a GC vial containing 4g of NaCl and immediately covering the vials with the crimp caps. The standards were then run in a sequence after which the average area of the individual THM standard peaks for all five levels was determined. With the Chemstation software, a linear calibration graph was then plotted against which the levels of trihalomethanes in the samples were measured.

STATIC HEADSPACE SAMPLING

This technique was used in analyzing the samples which involved the use of a closed sample container and a sampling system. After placing the sample matrix into the sealed sampling vial, the sample matrix was then heated for a specified time, during which the vial was also agitated (shaken) to help drive volatile compounds from the matrix and into the headspace volume. After a specified time, the vial was punctured, pressurized, and an amount of the headspace vapours were withdrawn and injected into the GC inlet. This was achieved by the use of the headspace auto sampler 7697A model and the conditions under which it was operated are all mentioned in the instrumentation section.

PRELIMINARY INVESTIGATION TO CONFIRM THE PRESENCE OF THMS IN BEER

As part of the research, a preliminary investigation was conducted to ascertain the presence or absence of THMs in beer samples. This was done by running two different samples as follows: the first sample was a normal beer sample prepared as indicated in the sample preparation and then run on the GC and the second sample was the same as the first but was spiked with a 1000µl of 1000µg/l trihalomethane calibration mix stock solution and then run on the GC. The chromatograms obtained from the two runs are shown in the as figures 1 and 2.

This investigation was very critical since it made it possible to identify each trihalomethane in relation to its retention time on the chromatographic column used (HP-5, 30 m × 0.32 mm × 0.25 µm) since different columns will give different retention times for the same compound.

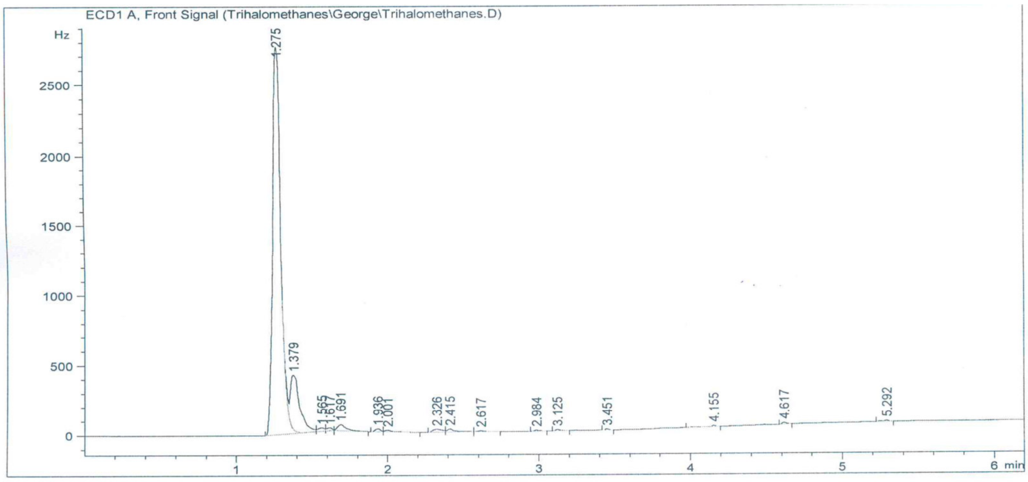

Figure 1: Chromatogram of unspiked beer

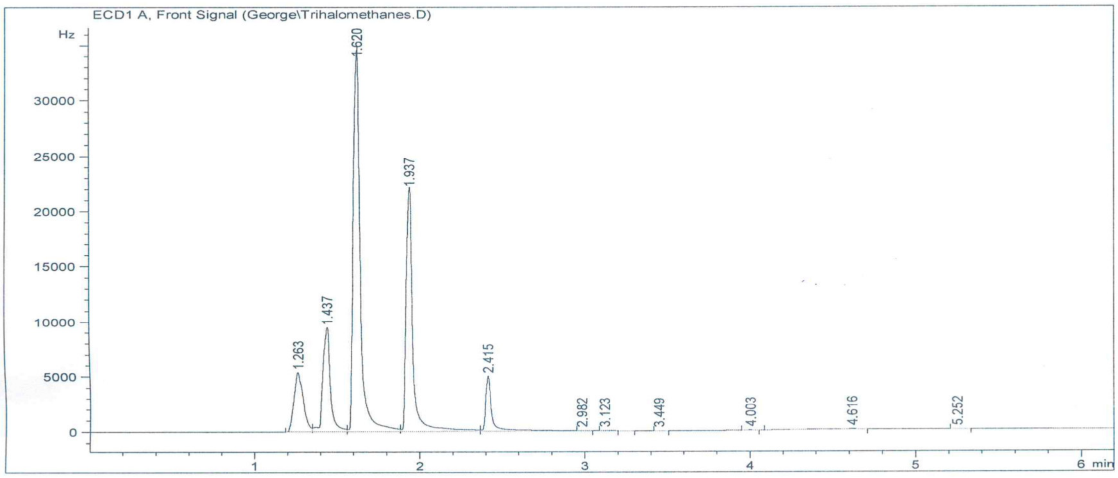

Figure 2: Chromatogram of beer spiked with 100ul of 1000ppb trihalomethane stock solution

FINDINGS

The results presented in the tables 1,2 3 below are for beer samples which were analyzed for the levels of trihalomethanes using a gas chromatograph equipped with an Electron Capture Detector (ECD) and a headspace auto-sampler.

A total of 48 lager beers were analyzed which comprised of six (6) different brands and eight (8) different batches from a brand. Brands ‘A’, ‘B’ and ‘C were products from the brewery X whilst Brands ‘D’, ‘E’ and ‘F’ were brands from brewery Y.

Table 1: Levels of chloroform and bromodichloromethane in 48 beers samples from 2 breweries in Ghana

BREWERY	Sample	$CHCl_3$(µg/L)			$CHCl_2Br$(µg/L)		
		Min	Max	Mean + SD	Min	Max	Mean + SD
X	A	ND	ND	ND	0.7526	0.8983	0.8070 ± 0.05
	B	ND	ND	ND	0.7526	0.8904	0.7956 ± 0.05
	C	ND	ND	ND	0.7526	0.81506	0.5826 ± 0.36
Y	D	0.3644	2.8379	0.6394 ± 01.11	1.0920	3.0157	1.6101 ± 0.71
	E	1.3771		0.1721± 0.49	0.9747	2.4858	1.1771 ± 0.53
	F	0.0722	20.1897	6.0843± 6.65	0.8630	2.3167	1.1282 ± 0.49

Table 2: Levels of dibromochloromethane and bromoform in 48 beers samples from 2 breweries in Ghana

BREWERY	Sample	$CHClBr_2$(µg/L)			$CHBr_3$(µg/L)		
		Min	Max	Mean + SD	Min	Max	Mean + SD
X	A	0.3667	0.4610	0.4204 ± 0.03	0.7742	0.9870	0.8200 ± 0.07
	B	0.3248	0.3940	0.3544 ± 0.02	0.7702	0.8564	0.8032 ±0.03
	C	0.2825	0.3590	0.2411 ± 0.15	ND	ND	ND
Y	D	0.3171	1.6218	0.9195 ± 0.40	0.5584	0.9768	0.7878 ± 0.12
	E	0.5015	1.3141	0.6709 ± 0.27	0.7041	0.7337	0.3604 ± 0.39
	F	0.3134	0.5700	0.3680 ± 0.09	ND	ND	ND

Table 3: Total trihalomethanes (TTHM) in 48 beers samples from 2 breweries in Ghana

BREWERY	Sample	TTHM
X	A	0.0259 ± 0.001
	B	0.0248 ± 0.001
	C	0.0121 ± 0.007
Y	D	0.0460 ± 0.017
	E	0.0305 ± 0.012
	F	0.0428 ± 0.030

Beer brewed by the 2 breweries is high gravity beer that is diluted to bottle specification using dilution liquor. Though some specific brands or batches did not record specific THM, TTHMs were detected in all beer sample analyzed, suggesting that the presence of THMs in the bottled products were traceable to the dilution liquor used in diluting the high gravity beer to sales specification. This fact is supported by an earlier research by Baxter (1999) who reported liquor used in the brewhouse loses most of these undesirable organic constituents during the boiling stage. However, the blending liquor, used to dilute beers brewed at a high gravity down to sales strength, is not heated and is therefore the source of THM in such beers.

A study by Montesinos(2014) in Spain reported higher concentration of 35µg/l for chloroform and 52µg/l for total trihalomethanes in two different samples of Soda. In that same study, beverages which were prepared by addition of larger volumes of treated water contained higher concentrations of trihalomethanes. This further confirmed the fact that the dilution liquor may be responsible for the introduction of THMs into the beer produced by the higher gravity approach.

Among brands 'A', 'B' and 'C'; the highest THM detected was bromoform with a mean concentration of 0.987 ± 0.05µg/l and the least detected was dibromochloromethane with a mean concentration of 0.282 ± 0.06 µg/l. chloroform was not detected in any of the brands A, B and C. On the other hand, chloroform was detected in brands 'D', 'E' and 'F' with one specific batch of F recording as high as value of 20.1879µg/l and an average value of 6.0843 ± 6.58µg/l for the eight samples. The least THM detected in brands 'D', 'E' and 'F' was chloroform with mean concentration 0.0722µg/l.

Morrison(1982) reported that of the four THMs that can occur in significant concentrations in chlorinated water, chloroform was detected most frequently and in the highest concentrations. however in this analysis chloroform was either not detected in the product and where it was detected it recorded the least concentration compared to the three order THMs. This observation could be attributed to the fact that the brewing houses have In-Plant Water Treatment Systems that is quite efficient in the removal of chloroform. Brewery X, in particular, can be said to have a 100% efficient chloroform removal system since chloroform was not detected in any of its products.

Wu et al (2006) reported a maximum level of 5.2µg/l total trihalomethanes whiles Montesinos and Gallego (2014) reported 52µg/l total trihalomethanes and these levels are far higher than the maximum level of 0.0460µg/l total trihalomethanes detected in this study

The total trihalomethane concentration reported in this study appears lower than that reported by Wu et al(2006) and Montesinos and Gallego(2014) where they used an older formula which is a simple summation of the levels of the individual THMs. However, in this study, total trihalomethanes was estimated by a new proposal by the W.H.O. (W.H.O., 2014) where the sum of the concentration of each individual trihalomethane divided by its guideline value was adopted.

The concentrations of THMs recorded were far below the recommended total daily intake (TDI) guideline values but the US-EPA estimates drinking 2 liters of water containing 100µg/l THMs every day for 70 years could result in 3 extra cases of cancer for every 10,000 people. By extension, if the batch of beer which contained 20.19µg/l of chloroform is used as a case study, consuming 5 bottles of volume 625ml a day will results in ingesting 100.95µg/l of chloroform a day; thereby putting one in the US-EPA estimation. Therefore heavy drinkers of beer containing THMs stand a high risk of developing cancer related health problems in the long term.

Conclusion

The concentrations or levels detected were below the World Health Organization's total daily intake (TDI) guideline values of 300µg/l, 60µg/l, 100µg/l and 100µg/l for chloroform, bromodichloromethane, dibromochloromethane and bromoform respectively in drinking water. Moreover the levels detected were also lower than that proposed by the US-EPA for drinking water which is 80µg/l. However, regular intake of beer with higher concentrations of trihalomethanes as it was observed in some of the samples can increase the risk of one being diagnosed of cancer related ailments in the longer future. On the whole, in order for these contaminants to have adverse effects on one's body, the person would have to consume large amounts of the breweries product at a time. An individual drinking this amount of beer would see far more health issues related to the consumption of a mass amount of alcohol, rather than the carcinogens found in these beers.

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